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Short Report

Disease Specific Biomarkers of Abdominal Aortic Aneurysms Detected by Surface Enhanced Laser Desorption Ionization Time of Flight Mass Spectrometry

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ABSTRACT

Introduction: Biomarkers have the potential to improve the clinical management of patients with AAA.
Report: A prospective, proteomics discovery study was undertaken to compare patients with AAA ($n = 20$) to matched screened controls ($n = 19$) for plasma protein expression. Surface-Enhanced-Laser-Desorption-Ionization Time of Flight Mass Spectrometry (SELDI ToF MS) coupled with Artificial Neural Networks (ANN) analysis identified six protein related diagnostic biomarker ions with a combined AUC of 0.89.
Discussion: This study discovered a signature plasma protein profile for patients with AAA and demonstrated that mass spectrometric based research for disease specific biomarker of AAA is feasible.

Introduction

Biomarkers may aid the diagnosis of AAA, predict progression and allow assessment of response to surgical and non-surgical treatments. Advances in mass spectrometry and statistical data analysis now allow high throughput screening of plasma for potentially useful biomarkers using a non-hypothesis based approach. The aim of this study was to use mass spectrometry to discover protein signatures that could discriminate AAA patients from healthy controls.

Report

Ethical approval for the study was granted by the Leicestershire, Northamptonshire and Rutland Research Ethics Committee (REC Study Reference # 6819). Written informed consent was obtained from all participants.

Twenty patients (median age 78.5 years, range 66–87) with AAA (median diameter 5.6 cm, range 3.8–8.2) and 19 ultrasound screened controls (median age 75 years, range 65–86) with normal aortic diameter (<2.5 cm) were prospectively recruited. All study participants were white European men. The exclusion criteria for the study were; any acute infective process, inflammatory condition or malignancy. Blood was collected at rest using standardized blood collection and plasma separation protocols (see supplemental material S2 for detailed methods). There was no freeze thaw cycle prior to final analysis. The weak cation exchange chromatographic surface (Protein Chip® CM 10 array from Bio-Rad Laboratories Inc, California) was used for plasma preparation for SELDI ToF analysis. The arrays were prepared according to the manufacturer's standard protocol with HEPES buffer using automated liquid handling (Biomek–FX from Beckman Coulter Inc, California). SELDI-Tof MS (PCS 4000 instrument, Ciphergen Inc. California) was used to generate spectral data in a single 6 h time period. Mass spectra were successfully obtained from all samples. The spectral data were analysed using a stepwise ANN analysis. A total of 11425 plasma protein ions were screened for association with AAA. At this stage principal component analysis was undertaken as a quality control measure which identified two outliers with aberrant spectra. These spectra were excluded from further analysis (1 AAA and 1 control). Further details of the process of ANN analysis are given in the supplementary material (S1). These analyses identified protein ions from the spectral data which could differentiate plasma samples from patients with AAA in a mixed population. The mass/charge ratio (m/z in Daltons) values for these ions were 992.3, 10710.7, 11249.6, 14465.1, 15201.7, and 13390.5 Da. The sensitivity

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and specificity of this classifier to discriminate AAA from controls were calculated at 85.0% and 81.4% respectively. A classifier consisting of these six ions showing (individually) a difference in expression between AAA and controls was identified. The area under the curve (AUC) for these potential biomarker classifiers (treated as single entities) was calculated as 0.89 by receiver operating characteristics (ROC) analysis (Fig. 1b). Internal cross-validation of the biomarker panel was undertaken on blinded data from the same study population. Testing this classifier on blinded samples (same study population) as a diagnostic test demonstrated that the majority of samples were correctly assigned (Fig. 1c). At a probability threshold of 0.5 only 5 incorrect assignments were seen (2 AAA, 3 controls).

Discussion

Multiple proteins (serum elastin peptides and plasmin–antiplasmin complexes, Matrix-degrading metalloproteinase 9,

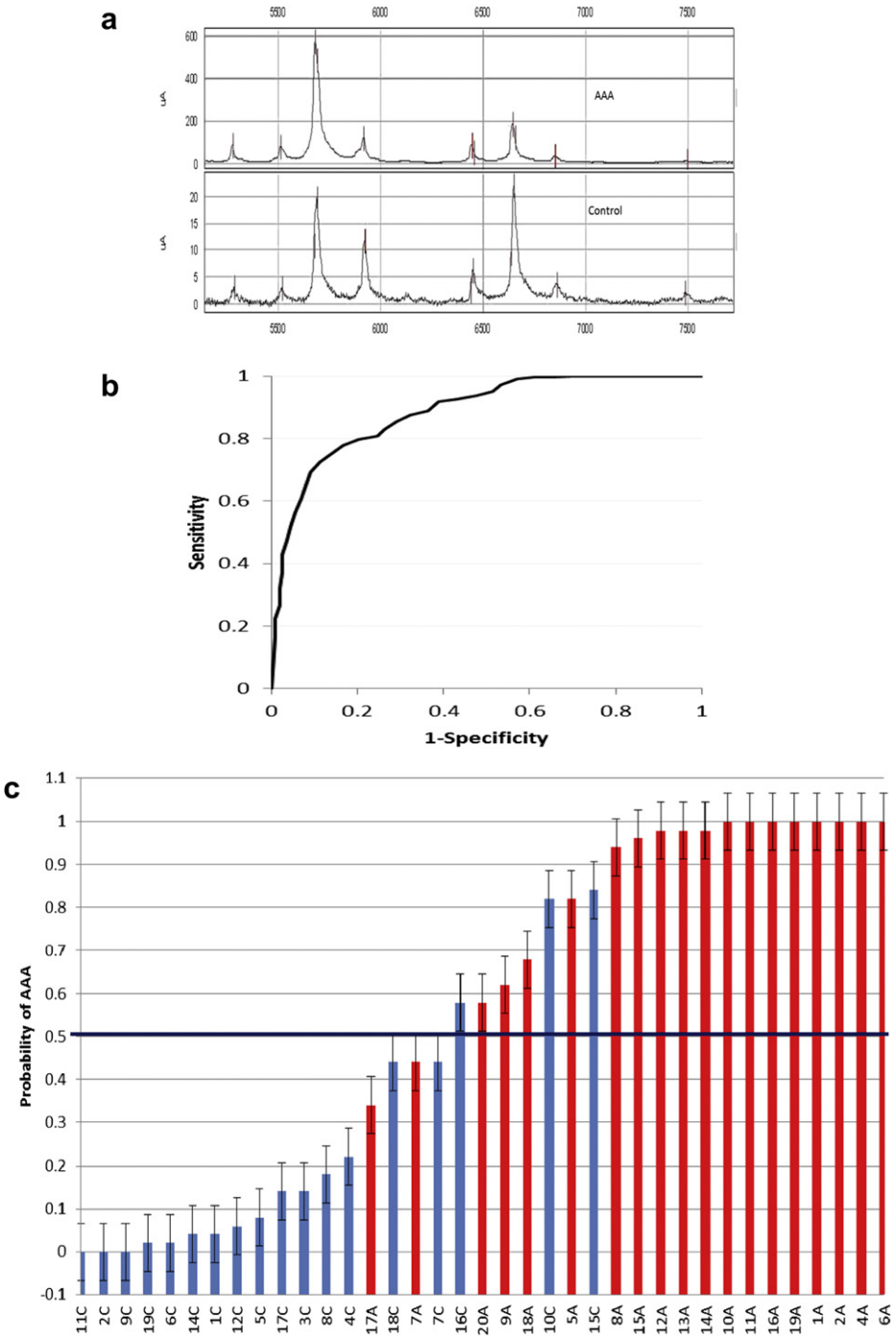

Figure 1. (a) Sample SELDI-Tof Mass Spectra of AAA and control. Signal intensity is shown on the *y*-axis (note variable scale) and mass to charge ratio on *x*-axis (UA = microamperes). (b) Receiver operating characteristic analysis for the six ion biomarker classifier. AUC = 0.89. (c) Cross Validation. The biomarker panel was tested against blinded data to discriminate cases (red bars) from controls (blue bars) At a cut-off of 50% probability (horizontal line) there were 5 incorrect assignments (2 AAA, 3 controls). The black error bars represent the associated standard error.

interferon-gamma, C-reactive protein, alpha 1-antitrypsin and lipoprotein (a), thrombin activation measured as APC-PCI and IL-6) have been investigated as biomarkers or risk factors for AAA. Although these proteins have been shown to be associated with AAA none have been independently validated or have found the potential for clinical use. Therefore further discovery studies to investigate other methodologies and new candidates are a valid avenue for further research.[1–3] This study has shown that a modern but relatively simple, automated high throughput proteomics technique can be used to study the plasma protein expression of AAA. This signature plasma protein profile can identify patients from a mixed population group.

In conclusion, this study has discovered a unique plasma protein expression for patients with AAA. The biomarker of AAA (classifier comprising of six ions) was found to be highly discriminatory for the disease. This differential expression merits further investigation as a risk stratification tool. In addition advanced proteomic techniques can be used for in depth analysis to discover proteins that are responsible for this differential expression and the pathological pathways that are dysregulated in patients with AAA.

Conflict of Interest/Funding

None.

Appendix A. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.ejvs.2012.04.018.

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